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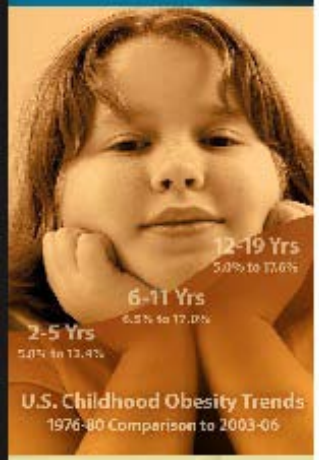
National Toxicology Program



NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

Breakout Group on

Bisphenol A (BPA)

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Crabtree Marriott Hotel
January 11-13, 20111

Bisphenol A Breakout Group Members

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What is the consistency, coherence, and strength of association of the evidence for or against an association between BPA and diabetes or obesity provided by the toxicological literature?

- There is a general lack of consistency in the data
- *In vitro* mechanistic data more strongly support an effect of BPA on glucose homeostasis and adipogenesis than on obesity
  - Increasing the number of adipocytes does not result in obesity without a change in energy balance

Deficiencies and/or limitations of current data

- Body weight is not a good measure of obesity
  - Some group support for ranking studies reporting body weight only as having lower utility than studies that report other metrics
- Fat mass, fat pads, and cellularity of adipocytes are important measures, and preferred over body weight alone
- Dietary differences in studies may contribute to inconsistencies in results
- Importance of studies of effects of exposure at other sensitive periods—e.g. prepubertal, perinatal when CNS centers regulating adipose mass are developing

Can limitations be addressed with additional animal studies?

- BPA exposures in various ER knock-out animal models will help address the role of ER mechanism and could help identify target tissues and target sites
- Humans are exposed to BPA throughout life, and animal studies that expose for a lifetime are needed
- Then may refine by limiting exposure to particular windows
- Humans are not exposed to BPA alone; animal studies could be designed to address mixtures, and the additional effects of various diets.
- Suggest use of BPA-free cages, bottles, and animals that have no history of BPA exposure.
- Control for litter effects

Can animal models be used to address susceptible populations (based on gender, life stage and health status)?

- Yes:
  - Can address key periods of development
  - Can address the influences of dietary variables and reflect actual differences in diet for different human populations (e.g., high fat diet)
  - Can use sensitive strains (sensitive to diabetes) to look at genetic susceptibility
  - Evaluate species differences (rat versus mice, primates)
  - Gender – include both sexes in studies

What key research gaps could be filled by further animal studies?

- It is most important to address the lack of consistency in available data
  - Endpoints: adipose deposition and distribution, BW, length; glucose homeostasis (glucose tolerance test, insulin tolerance test, and plasma insulin)
  - Importance of looking for effects across lifespan
  - Need to correlate exposures with plasma levels of BPA
  - Address variability in diet, route, timing, duration of exposure

What key research gaps could be filled by further animal studies?

- Source of BPA-purity is a concern; standardized source is optimal
- Identify specific targets: brain, muscle, fat, pancreas, liver, adipocytes, immune endpoints
- Non-human primate studies
- Epigenetic studies
- The data do not establish that BPA acts on obesity through ER
  - Postnatal knock out study on BPA is critical; tissue specific knock out
  - Evaluate potential non-ER mechanisms

What is the strength of the mechanistic data, including the *in vitro* studies and do they support biological plausibility?

- The available *in vitro* mechanistic data are limited
- Available data support a biological effect – particularly the effect on beta cells and adipocytes

Given what we know about the specific biology of the diseases, what are we missing in the way of animal experimentation, mechanistic experimentation, and *in vitro* endpoints for Tox21?

- The workgroup noted the breadth of endpoints demonstrated in Tox21/ToxCast™
- Workgroup did not discuss new assays in detail

Human studies – Current literature

- The current data from human studies of exposures to BPA show an association between BPA levels and self-reported diabetes. The two studies are somewhat inconsistent and the data are limited
- Of interest is the reported association of BPA levels with cardiovascular endpoints, (an aspect of metabolic syndrome)
- Three additional human studies have looked at the association between BPA and growth or body weight as endpoints and the findings were inconsistent
- Human data are too sparse to draw any meaningful conclusions

Human studies - What are the most useful indicators of exposure and health effect diagnosis?

- Exposure
  - BPA measurements in mothers and offspring are needed
  - Include measurement of conjugated and unconjugated BPA
  - Consider occupational sources of exposure
- Health effects
  - same as animal studies when possible; blood glucose, insulin, homeostasis model assessment (HOMA), MRI for adiposity

Human studies - What are the most important factors to include as adjustment variables?

- Age, sex, smoking, SES, diet, physical activity
- Look for interaction among variables; effect modification

Is it possible to explain the reported effects of BPA with what we know about classic estrogen receptors (α, β) and glycemic control/adiposity from the broader literature?

- ERα effects multiple pathways (such as LKB1/AMPK), decreasing obesity and improving insulin resistance
- Loss of estrogen receptor activity results in insulin resistance and obesity;
- BPA, as an ER agonist, may result in the same effect
- This appears inconsistent
- It may be explained by perinatal programming: estrogen programs glucose regulation/adiposity during this period

What if we consider other mechanistic activities that are being reported for BPA?

- Mechanisms of BPA action are not clear; however, it is clear that it is not just an estrogenic agent
- BPA has non-ER mechanisms
  - Anti-androgen at low doses
  - Affinity for ERR gamma
  - Effects not blocked with ICI-182 780; a gamma adrenergic receptor mediated mechanism proposed
- Is there evidence for non-receptor mediated, intracellular signaling pathways; alteration of enzymes in the steroidogenic pathway, etc.?

Is it possible to identify a positive control compound for *in vivo* studies that look at the effects of BPA on metabolic parameters? Might multiple control compounds be necessary to account for multiple mechanisms?

- If you don't know the mechanism(s) through which BPA acts, then you cannot identify a best positive control
- A strict estrogen agonist as a control may have limitations and assumes an estrogenic mode of action.
- It may not be sufficient to only use strong estrogen agonist (estriol may be better than E2)

Examples of mechanistic data supporting effect of low doses of BPA on glucose homeostasis

- *Ex vivo*
  - altered pancreatic alpha and beta cell signaling
  - inhibited release of adiponectin in mouse and human adipocytes
- *In vivo*
  - glucose intolerance, insulin resistance, postprandial hyperinsulin (injected dose of 100 µg/kg for 4 days in male 2 month old mice)
  - Altered glucose homeostasis in mice during pregnancy
  - Altered glucose homeostasis in older adult offspring following exposure of pregnant dams

Group recommends expanding discussion of:

- Androgen receptor (AR)
  - Add AR as mechanism of action for consideration
  - Explore vinclozolin, p'p'-DDE literature for AR antagonist effects
    - DDE human literature, cohort studies
  - Also look at pesticides that are weak ER agonists and AR antagonist
  - Anabolic steroids in foods

Group recommends expanding discussion of:

- Thyroid disruption
  - Need literature review for association of thyroid hormone and diabetes/obesity
  - Note ToxCast™ identified compounds

- Glucocorticoid disorders
  - Need literature review for association of glucocorticoid disorders and diabetes/obesity
  - Note ToxCast™ identified compounds